

Yeast Concentration, pH, and Fermentation Time on the Production and Concentration of Bioethanol Made from *Sargassum crassifolium* as a Renewable Energy Source

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Abstract: *Sargassum crassifolium* is one type of brown algae which has many benefits and has a high economic value. It has a relatively short harvest age. It is widespread in Indonesia's marine waters with high production potential, but the production is still largely from the harvest of natural supplies. *Sargassum* contains a lot of alginate polysaccharides that are used for food-beverage, cosmetics, and pharmaceutical industries. In addition, it also contains other types of polysaccharides namely cellulose (part of the cell wall), manitol (as stored carbohydrates) and fucoidan. The cellulose content in *Sargassum* ranges from 23.97 to 35.22%. Thus, it can be processed into a raw material in the production of bioethanol. The results of the research showed that the yeast concentration 3%, the medium pH 7, and fermentation time 72 hours gave the best results in increasing the production and content of bioethanol made from *Sargassum crassifolium*.

Keywords: Yeast, pH, Fermentation Time, Bioethanol, *Sargassum crassifolium*

In general, the energy needs in the world nowadays still depend on the fossil resources, especially oil, gas and coal. These natural resources have been formed thousands of years ago. The level of human consumption on the fossil energy is higher than the rate of the formation of this fossil energy. Whereas, these energy resources are included as nonrenewable resources, which means that if these resources are continuously consumed, at some point its availability in nature will be exhausted. If the use of fuel is continuously exploited, it is feared that there will be a fuel crisis that has a strong impact on people's quality of life as what we feel today (Fernandez *et al*, 2011; Lin *et al*, 2012; Radocaj and Diosady, 2014). The efforts to develop and utilize new and renewable energy sources are highly dependent on economic feasibility issues. This problem will be more apparent if the application is developed in developing countries. However, the application of certain technologies is still not economically feasible when applied in rural areas (3). Therefore, it is necessary to consider the appropriate development of the technology, so that the adaptation level can be higher for the rural and agricultural conditions in Indonesia.

It is time for the use of renewable energy sources such as biofuels or *bahan bakar nabati* (BNN) or bioenergy should be enhanced, replacing the fossil fuels which are diminishing. One example of liquid vegetable fuel is a substitute for gasoline named bioethanol. Bioethanol is a liquid produced from the fermentation process of vegetable materials containing chemical components of carbohydrate groups such as starch, starch, fiber, cellulose and others by microorganisms such as mold, yeast or bacteria through the process of conversion of chemical components into glucose which is then distilled into a chemical liquid called ethanol (Westman and Franzen, 2015). Ethanol is an organic compound consisting of carbon, hydrogen and oxygen, so that it can be seen as a derivative of hydrocarbon compounds having a hydroxyl group with the chemical formula C_2H_5OH . Ethanol or ethyl alcohol known as alcohol is colorless, odor-specific, flammable and evaporative liquid which can be mixed in water in all its comparisons and has premium resembling properties (Westman and Franzen, 2015; Zambare and Christopher, 2012).

Bioethanol can be produced from vegetable materials. The utilization of biological materials have been researched and developed to produce bioethanol such as: cassava, corn, sago, palm, sugarcane and grains which basically contain carbohydrate molecules that can be converted into bioethanol. Another unique and interesting thing about bioethanol is now being studied and made by using unused organic waste such as banana peel, sugar cane waste, sago pulp, the peel of sweet potato and corncobs and other organic waste. In addition, nowadays marine organisms are under the spotlight by the researchers by using seaweed (algae) as raw material for producing bioethanol, such as brown algae from *Sargassum species* (Wilkins and Atiyeh, 2011).

Research related to algae as one of the alternative bio-fuel material has been widely studied and utilized. Research results on the production of bioethanol made from *Sargassum* of the *S. duplicatum* speceis using fermenters, namely *Trichoderma Viridae* mold and *Pichia angophorae* ever yeast researched by Sari Nurbaya et al, by observing its optimum condition showed that the optimum time for fermentation for the bioethanol production is for 3 days at a temperature of 29 °C and pH of 4.17 with a growth rate of *Pichia angophorae* 0.48 produce rough ethanol 0.04 g/L, and the distillation process can raise the concentration of ethanol to 10.50 g/L. The study of algae as the material for bioethanol production and *Saccharomyces cereviceae* as biofermentor has also been investigated (Wilkins and Atiyeh, 2011; Younesi *et al*, 2005; Park *et al*, 2014). The research results show that the best condition for hydrolysis is on the concentration H_2SO_4 0.4 M (23, 051 mg / ml \pm 1.100) and 120 minutes (23.128 mg / ml \pm 6.069). During the fermentation process, the maximum bioethanol concentration is achieved at the incubation time 72 hours, which is at 0.0451% v/v \pm 0.0098.

MATERIALS AND METHODS

The preparation of Sargassum crassifolium sample

Sargassum crassifolium used was from the sea waters of Liang village. The preparation of *S. crassifolium* sample was done by collecting *S. crassifolium* as many as needed with scissors. After that, the samples were washed with fresh water, and then dried out under the sunlight for 12 hours. The dried *S. crassifolium* was cut into a size of 1 cm and then blended with a blender. The next step was to put the *S. crassifolium* sample which had been milled into an oven at a temperature of 50 °C for 4 hours (Perales *et al*, 2011; Percival *et al*, 2006; Perez *et al*, 2013).

Delignification

Delignification was carried out by weighing as much as 100 grams of *S. crassifolium* powder added with 500 ml of 2% NaOH into the Erlenmeyer. And then heated and stirred with a stirrer for 3 hours at a temperature of 80 °C. After that, the solutions were separated with a filter. The separated *S. crassifolium* powder was then rinsed with water (Percival *et al*, 2006; Perez *et al*, 2013).

Hydrolysis

The Hydrolysis process was done by, filtrate of the delignification results that had been added to the Erlenmeyer by adding a solution of HCl 21% up to 500 ml, and then the hydrolysis solution was put into an oven at a temperature of 80 °C for 3 hours.

The preperation of Starter

The starter used was bread yeast grown in growth substrate. The yeast used in this research was the commercial yeast sold in the market with the brand *pakmaya*. The growth substrate consisted of 1000 ml aquades added with 100 g of sugar (10% concentration) prepared in a beaker glass. After all the ingredients were included, and then it was initially homogenized with a *magnetic stirrer* and then sterilized using an autoclave at 121 °C for 15 minutes. The substrate was waited until it was cool. When the substrate was cool, approximately 30-33 °C, the yeast was put into the substrate, and then incubated into an incubator at a temperature of 30 °C for 8 hours. Much yeast in the starter making was adjusted to the amount of yeast concentration treatment, which was; 1%, 2%, and 3%.

Inoculation of starter

After the starter was incubated for 8 hours, the starter was ready to be inoculated in the fermentation substrate. The starter was included in the fermentation medium under aseptic conditions. The amount of starter included was 10%.

Fermentation

The fermentation process was done by filtering the filtrate from the hydrolysis process, and then the result of filtration in the form of liquid was added to erlenmeyer and added with NaOH or HCl until the pH became 5, 6 and 7 for pH variation, and then adding a starter as much as 300 ml in accordance with the starter concentration. The fermentation process was carried out according to the treatment time, ranging from 48 hours, 72 hours, and 96 hours.

Distillation

The testing of the alcohol level began with the distillation process. The fermentation results were distilled to separate ethanol from other solutions. The distillation was done at a temperature of 80 °C.

Testing of Ethanol concentration

The testing of ethanol concentration was carried out by using *Gas Chromatography* (GC) method. The results of the *Gas Chromatography* (GC) method was in the form of chromatogram, from which it can be known: the retention time, % area, and % high or concentration. The analysis using GC was done by injecting 1 µL sample. Then the profile of chromatograph sample was compared to *references standard* on GC-MS program by pressing *similary search* button.

RESULTS AND DISCUSSIONS

The use of biological materials has been extensively researched and developed in producing bioethanol such as cassava, maize, sago, aren, sugar cane and grains which basically contain carbohydrate molecules that can be converted into bioethanol. Another unique and interesting thing about bioethanol making is now being studied and made by utilizing unused organic waste such as banana peel, bagasse, sago pulp, sweet potato peel and corncoobs and other organic wastes. In addition, nowadays marine organisms are under the spotlight by the researchers by using seaweed as raw material for the production of bioethanol, one of which is brown algae of the *Sargassum crassifolium* species (Perales *et al*, 2011; Percival *et al*, 2006; Perez *et al*, 2013; Menetrez, 2014; Mohammadi *et al*, 2011; Phillips *et al*, 2014).

Cellulose in *Sargassum* ranges from 23.97 to 35.22%. The cellulose used was the derived product of carboxymethyl cellulose (cmc) which is widely used as stabilizer, thickener, and emulsifier material. High cellulose content in *sargassum* is one of the potential to be used as material for producing bioethanol. The research results showed that *S. crassifolium* could be fermented by using yeast to produce bioethanol as Table 1 below:

Table 1. The Effect of Yeast Concentration on the Production (ml) and the concentration of Bioethanol (%)

Yeast Concentration (%)	Bioethanol production (ml)	Bioethanol concentration (%)
1	46	8.643
2	50	9.693
3	65	10.443

Table 1 shows that the production and the concentration of bioethanol for 100 g dry weight *S. crassifolium* produced from each treatment with constant pH and fermentation time is different, with the lowest volume and the lowest concentration was obtained in the treatment of yeast concentration 1%, while the highest volume and the highest concentration of bioethanol was obtained in the treatment of yeast concentration 3%.

In addition to the treatment of yeast concentration, this research also used various pH media with the use of yeast concentration 1% and fermentation time 24 hours with the following results

Table 2. The Effect of pH Media on the Production (ml) and the concentration of Bioethanol (%)

pH Media	Bioethanol production (ml)	Bioethanol concentration (%)
5	46	8.643
6	53	9.168
7	70	9.943

Table 2 show that the production and the concentration of bioethanol for 100 g dry weight *S. crassifolium* produced from each treatment using constant yeast concentration and fermentation time are different, with the lowest volume and the lowest concentration were obtained at pH 5 treatment, while the highest volume and concentration of bioethanol were obtained at pH 7 treatment.

In addition to the treatment of yeast concentration and pH media, this research also used variations of fermentation time using constant yeast concentration and pH media with the following results

Table 3. The Effect of Fermentation Time on the Production (ml) and the concentration of Bioethanol (%)

Fermentation Time (Hours)	Bioethanol production (ml)	Bioethanol concentration (%)
24	46	8.643
48	56	11.081
72	67	14.043

Table 3 shows that the production and concentration of bioethanol for 100 g dry weight *S. crassifolium* produced from each treatment using constant yeast concentration and pH media are different, with the lowest volume and concentration were obtained on the treatment of fermentation time 24 hours, while the highest volume and concentration of bioethanol

The Effect of Yeast Concentration, Fermentation Medium pH, and Fermentation Time on Bioethanol Production (ml)

The use of *S. crassifolium* as a raw material in the manufacture of bioethanol has not been done. In fact, its carbohydrate content is the highest compared to the other chemical compositions. The carbohydrate content of *S. crassifolium* is 36.93% higher than the proteins and fats with 5.19% and 1.63% respectively. In general *S. crassifolium* has a fairly complete nutrient content. Chemically, seaweed consists of water (27.8%), protein (5.4%), carbohydrates (33.3%), fat (8.6%) crude fiber (3%) and ash (22.25%). In addition to carbohydrates, proteins, fats and fiber, seaweed also contains enzymes, nucleic acids, amino acids, vitamins (A, B, C, D, E and K) and macro minerals such as nitrogen, oxygen, calcium and selenium as well as micro minerals such as iron, magnesium and natrium. The amino acids, vitamins and minerals in seaweed reach 10 - 20 times more than the land plants. When compared between proteins, fats, and carbohydrates, the carbohydrate component is the biggest of all. Therefore, the *S. crassifolium* can be used as the raw material for bioethanol production (Younesi *et al*, 2005; Park *et al*, 2014; Perales *et al*, 2011; Percival *et al*, 2006; Perez *et al*, 2013).

By using the treatment of yeast concentration, fermentation medium PH and fermentation time, it was obtained that the treatment had an effect on the production of bioethanol with the best treatment (yeast concentration 3%, pH 7, and fermentation time 72 hours). The use of yeast in this research served as microorganisms that fermented glucose to become ethanol. Yeast contains *S. cereviceae* which has big potential in changing sugars into ethanol. *S. cereviceae* is known as baker's yeast which has the highest potential to ferment sugars into ethanol on a facultative anaerobic conditions. That the use of *S. cereviceae* can accelerate the fermentation of glucose to ethanol, and the higher the concentration of *S. cereviceae* used, the greater the bioethanol production. This is because it is influenced by the number of cells that perform the process of changing glucose into ethanol. As long as the nutrients in the medium are available, the corresponding microorganisms will continue to reform and will end as the nutrients in the medium decrease (Richana, 2011; Azizah, 2012).

In addition to the use of yeast, the treatment of fermentation media pH of the fermentation medium will have an effect on the production of bioethanol. pH is the condition of acid-alkali medium of a microorganism that can affect the growth (cell division activity) of a particular microorganism. That pH plays an important role in the growth of fermentation microorganisms. pH is related to the degree of acidity of the medium that will determine the activity of microorganisms in addition to the availability of nutrients. The best pH in the combination treatment was 8. This is not in line with the opinion of Oura that the best pH for the growth of *S. cereviceae* ranged from 4.0 to 4.5. The pH difference between the one found by the researcher and the one found by the

previous researcher might be because the previous research did not use variation of treatment with other factors. The previous researcher only used one independent variable factor, which was pH. While this research used 3 combined factors, namely yeast concentration, medium pH, and fermentation time. This treatment difference is suspected to have an effect the needs of pH values by *S. cereviceae* contained in yeast.

In addition to the factors of yeast concentration and fermentation medium pH, fermentation time is one combination treatment that gives effect to the production of bioethanol by using *S. crassifolium* as a raw material. The fermentation time that gave the best results was 72 hours, which means that the logarithmic phase took place during that time. Logarithmic phase is the fastest growing phase experienced by microorganisms because of the availability of more nutrients than the presence of microbial cells. The large amount of nutrients results in the large amount of microbial energy to break down glucose into ethanol. Ethanol production is influenced by the fermentation time, where the fermentation time is related to the logarithmic time possessed by microbes to be in large quantities in remodeling glucose into ethanol. If the fermentation time is too long, the ethanol production can be reduced due to the occurrence of microbial cell death caused by lack of nutrients or intoxication of CO₂, which is a byproduct of the anaerobic fermentation process (Cruz *et al*, 2014; Sari, 2014; Ariyani, 2013; Richana, 2011).

The Effect of Yeast Concentration, Fermentation Medium pH, and Fermentation Time on Bioethanol concentration (%)

The amylum, which is a complex molecule composed by glucose monomers and linked by glycosidic bonds, can be broken down into glucose by the treatment of acid compounds or the addition of enzymes. The addition of acid compounds or the use of enzymes serves to break the glycosidic bonds that connect the glucose units. The glucose, formed from the amylum break down process, will then be converted into alcohol by microbes under anaerobic conditions. During the fermentation process, carbondioxide was produced, in addition to alcohol (Fernandez *et al*, 2011; Lin *et al*, 2012; Radocaj and Diosady, 2014; Westman and Franzen, 2015).

Any raw materials used in the manufacture of bioethanol will obtain different results, either from volume or concentration of ethanol. This is because of the high levels of amylum or glucose that decompose from amylum by enzyme activity or hydrolysis by using acid compounds. The volume reflects the amount of ethanol produced in one ml that can be measured after the distillation process, while the concentration reflects the content of ethanol in percentage which shows the amount per 100 ml sample. The formation of bioethanol from the raw material containing amylum, such as *S. crassifolium* is influenced by several factors: temperature, pH, yeast concentration, fermentation,

sugar, yeast nutrients, concentration of acid compound, and the concentration of the enzyme (Ariyani, 2013; Richana, 2011; Azizah, 2012).

The research results by using 3 variables, namely: yeast concentration, fermentation medium pH, and fermentation time show that it has an effect on the ethanol concentration produced. The highest ethanol concentration was obtained in the treatment (yeast concentration = 3 g, pH = 7, and fermentation time = 72 hours). The results of this research are in line with the opinion by Ria Restu and Anastasia that yeast concentration 3% produced the highest ethanol concentration of the raw materia of *Kepo* banana (*pisang kepo*) was 7.0774%. Yeast is a fermenting starter because it contains *S. cereviceae* which can break down glucose into ethanol. During the process fermentation, *S. cereviceae* contained in the yeast will use glucose as a source of nutrients and break down glucose into ethanol and carbon dioxide. The higher the yeast concentration used, it will affect the microbial interactions in using nutrients in the fermentation medium, so that it will affect the ethanol produced (Richana, 2011; Azizah, 2012)

In addition to yeast concentrations, medium pH is also a variable that affects the concentration of ethanol produced in the fermentation of glucose from remodelling amyllum *S. crassifolium*. pH is an acid-alkali condition of the fermentation medium associated with the growth activity of microorganisms. too low (acid) or too high (alkali) pH can lead to microbial cell death. High mortality rates of microorganisms will affect the speed of fermentation, because the number of microbes will decrease in fermenting glucose into ethanol. The research results showed that the best pH level of ethanol was 7. When compared with the relevant research results, it shows that the best ethanol concentration is by using a medium pH under acidic conditions (pH = 3 - 4) for raw materials such as banana peel, durian seed, and cassava. The research results found were not in line with the previous research results because this research found that the best pH is in neutral condition. This is presumably due to the differences in the raw materials used that require a higher pH value when compared with other organic materials.

CONCLUSION

1. The use of yeast concentration has an effect on the increase in volume (ml) and the concentration (%) bioethanol made from yeast *S. crassifolium* with the best yeast concentration 3%
2. The pH variation has an effect on the increase in volume (ml) and the concentration (%) of bioethanol made from *S. crassifolium* with the best pH 7
3. The fermentation time has an effect on the increase in volume (ml) and the concentration (%) of bioethanol made from *S. crassifolium* with the best fermentation time 72 hours

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